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Distribution of virulence factors in ESBL-producing *Escherichia coli* isolated at the environment, livestock, food and human interface

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Abstract

In this study, extended-spectrum ß-lactamase (ESBL)-producing *E. coli* isolates recovered from the following sources were characterized with regard to the occurrence and distribution of uropathogenic and enteric pathogenic virulence factors: surface waters (rivers and lakes, n=60), the intestines of freshwater fish (n= 33), fresh vegetables (n=26), retail poultry meat (n= 13) and the fecal samples of livestock (n= 28), healthy humans (n= 34) and primary care patients (n=13). Among the 207 isolates, 82% tested positive by PCR for one or more of the virulence factors (VF) that predict uropathogenicity, *TraT*, *fyuA*, *chuA*, PAI, *yfcv* or *vat*. Uropathogenic *Escherichia coli* (UPEC) were detected in each of the analyzed sources. Regarding virulence factors for intestinal pathogenic *E. coli*, these were found more rarely and predominantly associated with the aquatic environment, with *aggR* (EAEC) found in isolates from surface waters and STp (porcine heat stable enterotoxin) and LT (heat-labile enterotoxin) associated with isolates from fish. Aggregate VF scores (the number of unique virulence factors detected for each isolate) were lowest among isolates belonging to phylogenetic group B1 and highest among group B2. Clustering of the isolates by phylogenetic group, multilocus sequence type (MLST) and ESBL-types revealed clonal overlaps of A:ST10(CTX-M-1) and D:ST350(CTX-M-1) between the sources of livestock, poultry meat and healthy humans, suggesting livestock, in particular poultry, represents a potential reservoir for these particular UPEC clones. The clones A:ST10(CTX-M-55) and B2:ST131(CTX-M-27), harbouring uropathogenic virulence factors were significantly associated with fresh vegetables and with fish, respectively. Further clonal complexes with source overlaps included D:ST38(CTX-M-14), D:ST69(CTX-M-15), D:ST405(CTX-M-15) and D:ST648(CTX-M-15), which were found in surface water and healthy humans. Identifying potential reservoirs of UPEC in the environment, animals, food and humans is important in order to assess routes of transmission and risk factors for acquiring UPEC.

Keywords

Uropathogenicity, Enterobacteriaceae, CTX-M, clones, environmental sources.

1. Introduction

Escherichia coli is a bacterial species of multitudinous characteristics that occurs naturally in the digestive tract of humans and warm-blooded animals. Apart from non-pathogenic commensal isolates, two subdivisions of *E. coli* are, by virtue of their acquisition of virulence factors (VF), etiological agents of intestinal or extraintestinal diseases.

One first major group of pathogenic *E. coli* causes characteristic symptoms of gastrointestinal disease and consists of the pathotypes enteropathogenic *E. coli* (EPEC), shiga toxin-producing *E. coli* (STEC) and its subgroup enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusively adhesive *E. coli* (DAEC). (Nataro & Kaper, 1998). A second major group of pathogenic *E. coli* cause infections outside the gastrointestinal system and are termed extraintestinal pathogenic *E. coli* (ExPEC). This group includes avian pathogenic *E. coli* (APEC), which causes respiratory tract infections and septicaemia in poultry and uropathogenic *E. coli* (UPEC) (Kaper et al., 2004). Principally, the human intestinal tract is thought to be the primary reservoir for UPEC from where it can disseminate to the urogenital tract, causing in an ascending manner, urinary tract infections (UTIs) (Pitout 2012; Singer 2015).

Virulence factors are distributed unequally among commensal and pathogenic *E. coli*, enabling a classification according to phylogenetic group (Clermont et al., 2000). Thereby, most commensal stains belong to phylogenetic group A or B1, and extraintestinal pathogenic strains, which possess more VF than commensal strains, are assigned to phylogenetic groups B2 or D. Whereas for enteropathogenic *E. coli* each pathotype can be characterized and related to disease symptoms by its specific combination of VFs, (Kaper et al., 2004), there exists to date no concrete set of virulence factors for defining an *E. coli* as ExPEC or for distinguishing ExPEC subgroups from one another (Singer 2015). Although a basic virulence gene profile exists for both UPEC and APEC, VFs that are specific to UPEC and that can clearly distinguish it from APEC have not yet been identified (Wiles et al., 2008). Some studies therefore state that some pathogenic as well as non-pathogenic strains in domestic bird populations represent potential UPEC strains in humans (Danzeisen et al., 2013; Johnson et al., 2003; Maluta et al., 2014).

UTIs are among the most frequent human bacterial infections, and constitute a major global burden of disease (Marrs et al., 2005; Totsika et al., 2012). Consequently, the emergence during the last two decades of UPEC harboring antimicrobial resistance genes is a particular threat to human health (Pitout 2012). Many multidrug resistant *E. coli* strains that are commonly isolated from UTIs belong to specific worldwide endemic clones and have been detected in surface waters and water-related environments (Amos et al., 2014; Tausova et al., 2012; Zurfluh et al., 2013). These clones include the multidrug resistant, extended-spectrum ß-lactamase (ESBL)-producing *E. coli* B2:ST131 or the trimethoprim-sulfamethoxazole resistant *E. coli* Clonal Group A (CGA), a clone that clusters within phylogroup D: ST69 (Totsika et al., 2012). Identifying further potential reservoirs of these and other virulent ExPEC clones may help understand the way they spread throughout the environment.

The purpose of this study was to determine the occurrence and distribution of virulence genes in a collection of ESBL-producing *E. coli* isolates originating from a broad range of environmental, food, animal and human sources. These sources included rivers, lakes, freshwater fish, vegetables, livestock, retail chicken meat, healthy humans and primary care patients.

2. Materials and Methods

2.1. Strain collection

The collection of ESBL-producing strains consisted of 60 isolates from rivers and lakes in Switzerland (Zurfluh et al., 2013); 33 strains from the intestines of freshwater fish (Abgottspon et al., 2014a); 26 isolates from different types of fresh vegetables (basil, beans, bitter cucumber cha-om, coriander, chilli, curry leaves and okra) imported to Switzerland from the Dominican Republic, India, Thailand and Vietnam (Zurfluh et al., 2015); isolates from fecal samples of chicken (n=6), pigs (n=3), lamb (n=1) and cattle (n=1) collected from healthy animals entering the slaughterhouses (Geser et al, 2012); 17 samples originating from a longitudinal sampling study at 3 different broiler chicken farms distributed throughout Switzerland (Zurfluh et al., 2014); strains obtained from

poultry meat (n=13) (Abgottspon et al., 2014b); strains originating from fecal samples of healthy humans (n=34) or from fecal swabs of primary care patients (n=13) in Switzerland (Geser et al., 2012; Nüesch-Inderbinen et al., 2013a). Sources and identities of all strains, as well as isolation dates are indicated in Figure S1.

2.2 Virulence factor genes

DNA from *E. coli* isolates was extracted by a standard boiling procedure and all 207 isolates were screened by PCR for six markers of virulence associated with UPEC and eight marker genes for IPEC. The UPEC marker genes and the EAEC-specific gene *aggR* were amplified by conventional PCR using primers and conditions described previously for *traT*, *fyuA* and PAI (Johnson & Stell, 2000), *chuA* and *yfcv* (Spurbeck et al., 2012), *vat* (Ewers et al., 2005) and *aggR* (Boisen et al., 2012), respectively.

The IPEC virulence factors *eae* (EPEC), STh, STp and LT (ETEC), *stx1* and *stx2* (STEC) and *ipaH* (EIEC) were detected by real time multiplex PCR (Light Cycler) using QuantiFast Multiplex PCR Kit, (Qiagen, Hombrechtikon, Switzerland), and primers and cycling conditions according to the guidelines of the European Union Reference Laboratory for *E. coli* (EU Reference Laboratory for *E. coli*, 2013).

The aggregate VF score was defined as the number of unique UPEC-VF detected for each isolate, counting the PAI marker as one. Such molecular characteristics predict the extraintestinal virulence potential of an *E. coli* isolate *in vivo* (Johnson et al., 2006).

2.3. Multilocus sequence typing

For multilocus sequence typing of *E. coli* isolates, internal fragments of the seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA*, and *recA*) were amplified by PCR from DNA, as described by Wirth *et al*. (Wirth et al., 2006). Sequencing of the amplification products was performed by Microsynth (Balgach). Sequences were imported into the *E. coli* MLST database website (http://mlst.ucc.ie/mlst/dbs/Ecoli) to determine MLST types.

Alleles and STs that had not been previously described were assigned new designations by the curators of the database.

2.4. Phylogenetic classification

DNA from *E. coli* isolates were subjected to triplex PCR targeting the *chuA* gene, the *yjaA* gene and an unspecified DNA fragment termed TspE4.C2, as described previously (Clermont et al., 2000). Isolates were classified as belonging to one of the four phylogenetic groups A, B1, B2 or D, whereby group A and B1 typically contain commensal *E. coli* strains while groups B2 and D consist of virulent extra-intestinal strains (Johnson et al., 2001).

Multilocus sequence typing and phylogenetic classification were performed on isolates from the strain collection that had not yet been characterized to this regard. Thus, 50 of the 60 isolates from rivers and lakes (Zurfluh et al., 2013) and 29 of the 34 isolates from healthy humans (Geser et al., 2012) were additionally typed.

2.5. Statistical analysis

Comparisons of proportions of virulence genes and proportions of endemic clones within the sources were performed by Fisher's exact test in a series of individual pairwise comparisons using 2x2 tables where each characteristic was determined as present or absent. The significance criterion was set at $p < 0.05$. Calculations were performed using the VassarStats website for statistical computation (http://www.vassarstats.net).

3. Results and Discussion

3.1 Distribution of virulence genes throughout the sources

E. coli harboring uropathogenic virulence factors (UPEC) were detected throughout the sources (Figure S1).Overall, 82% of the isolates tested positive for one or more markers of uropathogenic virulence. Among the 207 *E. coli* isolates, the prevalence of individual VF genes ranged from 0% (*vat*, *stx1*, *stx2*, *ipaH*) to 55% (*TraT*, a lipoprotein involved in serum resistance) (Table 1). Among the sources, the distribution of *TraT* was distinguished by a significantly lower prevalence for isolates from healthy humans compared to all other sources, (p= 0.0001, OR 0.1945, CI 0.08-0.45).

The genes *chuA* and *fyuA* occurred at significantly lower rates in the isolates from the livestock source (p=0.00242, OR 0.3452, CI 0.1336-0.8924 and p< 0.0001, OR 0.1014, CI 0.03-0.35, respectively). Further, PAI (pathogenicity island) was significantly associated with isolates from healthy humans (p < 0.0001, OR 8.9728, CI 308-21) and vegetables (p=0.0008, OR 0.0723, CI 0.009-0.54). Hence, the presence or absence of PAI may be an important characteristic of a putative zoonotic or environmental strain in terms of potential transmission to humans.

The overall median aggregate VF scores (and ranges) of the isolates were the following: from surface water VF 1 (0-5), from fish 2 (0-5), from vegetables 1 (0-5), livestock 1 (range 0-5), retail meat 1 (1-5), healthy humans 2 (0-5) and primary care patients 3 (0-5).

The highest prevalences of VF were observed in isolates from primary care patients. Accordingly, the mean aggregate VF score was highest among this group. However, the large range of aggregate VF scores show that each source is contaminated with highly virulent pathogenic *E. coli.* With regard to *fyuA* and *chuA* which are associated with a large mean of other VF genes and have been described as predictors of UPEC (Spurbeck et al., 2012), a total of 72 (34.8%)isolates containing both these genes may be considered UPEC. These putative UPEC isolates showed the following distribution throughout the sources: surface water, 20 of 60 isolates (33.3%); fish, 17 of 33 isolates (51.5%); vegetables, 5 of 26 isolates (19.2%); livestock 3 of 28 isolates (10.7%); retail meat, 4 of 13 isolates (30.8%); healthy humans, 15 of 34 isolates (44.1%); and primary care patients, 8 of 13 isolates (61.5%)."

It is known from previous studies that uropathogenic *E. coli* can survive wastewater treatment and are released into surface waters (Anastasi et al., 2010). Pollution of water and the detection of uropathogenic isolates in freshwater fish destined for human consumption is of major concern. Guzman and collaborators (Guzmán et at., 2004) have shown that there is positive linear relationship between the concentration of *E. coli* in the digestive tract and the edible tissue of freshwater fish. Consequently, freshwater fish from polluted surface waters must be considered a potential risk factor for acquiring UPEC by handling or by cross-contamination during preparation. Currently, much attention is focused on the contamination of fresh produce with intestinal pathogens (Brandl & Sundin, 2013), but little is known about the risk of acquiring UPEC from fruit and vegetables.

The samples analyzed in this study originated from India, Thailand, Vietnam or the Dominican Republic, where wastewater without, or with insufficient treatment is commonly used for horticultural production (Zurfluh et al., 2015). Our data show that 17 of 26 (65.4%) of the vegetable isolates harbored one or more uropathogenic VF. Although the presence of a single VF gene is not sufficient to lable an isolate UPEC, these results highlight the need to broaden the focus on food-borne UTI to include food of non-animal origins.

Regarding virulence factors for intestinal pathogenic *E. coli*, these were predominantly associated with the aquatic environment, with *aggR* (EAEC) found in isolates from surface waters and STp and LT associated with isolates from fish (Table 1). "As expected, *aggR* was not detected among isolates from healthy humans, since individuals with intestinal infections were not included in this group. Although healthy humans may be carriers of EAEC, *aggR* is significantly associated with diarrhoeagenic strains (Nüesch-Inderbinen et al., 2013b).

3.2. Distribution of virulence genes among the phylogenetic groups

In accordance to their phylogenetic characteristics, the aggregate VF scores and ranges were low in group A (median 1, range 0-3) and group B1 (median 1, range 0-2) and followed an ascending gradient from through group D (median 3, range 1-5) to group B2 (median 5, range 2-5). Although classically defined as commensal (Clermont et al., 2000), 67.7% and 71.7% of the isolates of phylogenetic groups A and B1, respectively, harbored one or more VF marker (Table 2). Of the group A isolates, 33.9% harbored *fyuA*, which is described as an excellent predictor gene for a large number of other VF (Spurbeck et al., 2012). Furthermore, the pathogenicity island PAI detected in 19.4%, and 16.6% of group A and B1, respectively, includes two *pap* operons encoding P fimbriae and the *hly*CABD hemolysin gene cluster which are characteristics of highly virulent pyelonephritic *E. coli* (Kao et al, 1997). With regard to uropathogenicity, *E. coli* of the A and B1 group appear to be pathotypically heterogeneous.

3.3. Distribution of pandemic clones and other sequence types among the sources.

Phylogenetic groups within each source were stratified by clonal complex and VF scores were assigned to major endemic clones.

Clones were further stratified according to ESBL-types in order to determine their prevalence within each source and to identify clones with host source overlap. An overview is given in Table S1.

Clonal overlaps were detected for A:ST10(CTX-M-1), with prevalences markedly increased in the livestock source (chicken) and significantly associated with the healthy human source (p= 0.0299, OR 4.0887, CI 1.2-13.8). Previous studies identifying poultry meat as a possible zoonotic reservoir of UPEC are supported by the results of this study with regard to clone A:ST10 harboring CTX-M-1, which is a poultry-associated ESBL (Abgottspon et al., 2014b; Leverstein-van Hall et al., 2011; Zurfluh et al., 2014). This finding is a further strong indication, albeit no definite proof, of transmission from poultry to humans. By contrast, clone A:ST10(CTX-M-15), which included one isolate harboring the EAEC marker gene *aggR*, was significantly associated with water sources (p= 0.047, OR 4.3636, CI 1-19). Previously, multidrug resistant clone A:ST10 containing *aggR* and other EAEC marker genes has been implicated in an outbreak in Copenhagen (patients with UTIs), for which no common food source was identified (Olesen et al., 2012). Hence, the aquatic environment appears to be an important reservoir of at least some UTI-associated strains. Further IPEC-associated VFs STp (ETEC) and LT (ETEC), were found associated with isolates from freshwater fish (p=0.0248 and 0.0248, respectively, both with OR and CI = ∞).

Clone A:ST10(CTX-M-55) was detected exclusively in and significantly associated with, isolates originating from vegetables, all of which had been imported from Asia to Switzerland (Zurfluh et al., 2015). CTX-M-55 is an ESBL variant that is found increasingly in Asian regions (Xia et al., 2014). The emergence of these clones in imported food exemplifies the impact of global trade on putative UPEC reservoirs. Future monitoring of this specific clone may offer the possibility to pinpoint future transmission events, since so far it has not been detected in other sources in Switzerland.

Isolates belonging to phylogenetic group B1 were significantly associated with the livestock source (p=0.0001, OR 18.889, CI 7-51) and consisted in particular of B1:ST1056(CTX-M-1) isolates from healthy chicken and from chicken meat (Figure S1, Figure 1 and Table S1). One sequence type belonging to B1:ST446(CTX-M-1) overlapped between livestock and healthy humans and was observed in one isolate each, respectively (Figure S1).

Multiple sources shared isolates belonging to B1:ST155, whereby B1:ST155(CTX-M-14) and B1:ST155(CTX-M-15) were restricted to the aquatic environment and vegetable sources, while B1:ST155(CTX-M-1) was detected in vegetable and livestock sources and was associated significantly with the chicken meat source (p=0.0474, OR 8.6364, CI 1.4-52).

The globally dominant pathogenic clone B2:ST131 was found most prevalently in water, in fish, and in primary care patients. This clone is mainly associated with hospital and community-acquired infections in humans (Rogers et al., 2011; Singer, 2015). In particular, B2:ST131(CTX-M-15) was not found in livestock, retail meat or healthy humans. This finding contrasts with previous studies that reported food-borne origins of this clone (Manges & Johnson, 2012). Likewise, B2:ST131(CTX-M-14) was not found in livestock or chicken meat, but, by contrast to B2:ST131(CTX-M-15), in healthy humans. B2:ST131(CTX-M-27) was isolated at a markedly increased rate from the aquatic environment (3 isolates/5%), including fish (p=0.0379, OR 4.6621, CI 1.2-18.4), and also from primary care patients (2 isolates/15.4%). This is supportive of previous studies that detected this clone in waterfowl and clinical settings (Micenková et al., 2014; Tausova et al., 2012; Zurfluh et al., 2013).

Clone B2:ST95 which is known to be shared between APEC and human ExPEC (Maluta et al., 2014) was detected in one healthy human.

Clones observed among phylogenetic group D included D:ST38, D:ST69, D:ST405 and D:ST648 (Table S1). They were detected at low rates in water, fish, vegetables and humans,

but not in livestock or retail meat. *E. coli* D:ST648(CTX-M-15) has been described in companion animals and horses, and is proposed as a novel extraintestinal clone (Ewers et al., 2014). In this study it was found significantly associated with the healthy human source (p=0.015, OR 4.6621, CI 1.6-35.5). This may indicate its anthropogenic origin.

The only clone from group D shared by isolates from livestock (pig), chicken meat and healthy humans was D:ST350. This clone has been detected in APEC causing salpingitis and peritonitis and is also associated with UTI in humans (Pires-dos-Santos et al., 2013). Possibly, D:ST350 (CTX-M-1) represents a zoonotic genotype and food animals other than poultry, i.e. pork, may constitute a reservoir.

Clearly, future investigations should be undertaken to clarify whether ESBL-producing UPEC clones detected in healthy individuals are also associated with UTI in diseased humans.

4. Conclusions

Pathogenic *E. coli* is a major burden of disease worldwide. This study identifies potential reservoirs of pathogenic, ESBL-producing *E.coli* in the environment, animals, food and humans. *E. coli* harboring virulence factors that predict uropathogenicity were detected throughout the analyzed sources. Overall, 82% of the isolates tested positive for one or more virulence factors. Source overlaps between the aquatic environment, livestock, retail meat and healthy humans were noted for the clone A:ST10(CTX-M-1). Overlaps between the aquatic environment and healthy humans included clones B2:ST131(CTX-M-14), D:ST38(CTX-M-14), D:ST405(CTX-M-15) and D:ST648(CTX-M-15). Further, livestock, retail meat and healthy humans shared clone D:ST350(CTX-M-1).

The simultaneous analysis of different potential reservoirs of UPEC contributes to current understanding of the characteristics of multidrug resistant, uropathogenic *E. coli* at the interface between multiple sources.and may be useful for assessing potential risk factors for UPEC infection and for future studies aimed at determining the directionality of the dissemination of pathogenic *E. coli.*

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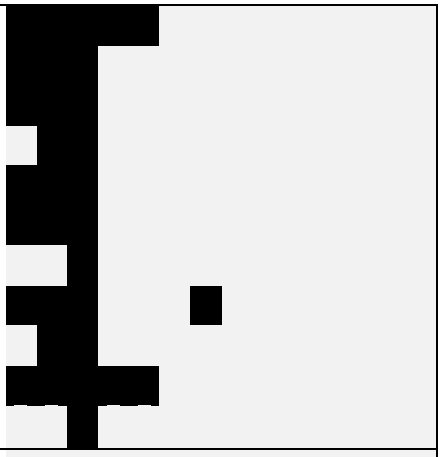
6. Figures

Source/Year(s) of isolation	Sample ID	ESBL	Phylo-group	CC	ST	Virulence factor (*E. coli* pathotype)												
						TraT (UPEC)	*fyuA* (UPEC)	*chuA* (UPEC)	PAI (UPEC)	*yfcv* (UPEC)	vat (UPEC)	*aggR* (EAEC)	*eae* (EPEC)	STh (ETEC)	STp (ETEC)	LT (ETEC)	*stx1* (STEC)	*stx2* (STEC)	*ipaH* (EIEC)
Surface water (n=60)/2012	OW4 ESBL SK1	CTX-M-15	A	10	10		■					■						
	OW8 ESBL SK1	CTX-M-3	A	10	34		■					■						
	OW29 ESBL	CTX-M-1	A	10	48													
	OW60 ESBL SK2	CTX-M-15	A	10	617	■	■		■									
	OW61 ESBL SK2	CTX-M-15	A	10	227	■	■											
	OW80 ESBL SK1	CTX-M-15	A	10	617	■	■		■									
	OW95 ESBL SK1	CTX-M-15	A	10	10	■												
	OW96 ESBL SK2	CTX-M-3	A	10	10	■												
	OW3 CRE SK1	CTX-M-15	A	23	410		■											
	OW16 ESBL SK2	CTX-M-15	A	23	410													
	OW29 ESBL SK1	CTX-M-15	A	23	90	■												
	OW55 CRE SK1	CTX-M-15	A	23	410													
	OW65 ESBL SK2	CTX-M-1	A	165	189													
	OW1 ESBL SK2	CTX-M-55	A		5048	■												
	OW8 CRE SK2	CTX-M-15	A		3224	■	■		■									
	OW19 ESBL	CTX-M-15	A		1408													
	OW34 ESBL SK1	CTX-M-14	A		3610		■											
	OW48 E SK1	CTX-M-1	A		744													
	OW55 ESBL SK1	SHV-12	A		710	■	■											
	OW60 ESBL SK1	CTX-M-14	A		2705													
	OW64 ESBL SK1	CTX-M-15	A		1312									■				
	OW68 ESBL SK1	CTX-M-15	A		1294													
	OW76 ESBL SK2	CTX-M-14	A		2172													
	OW77 CRE SK1	CTX-M-15	A		5053	■			■									
	OW78 ESBL SK1	CTX-M-15	A		361													
	OW60 CRE SK2	CTX-M-79	B1	101	101	■												
	OW54 ESBL SK2	CTX-M-55	B1	155	58	■	■											
	OW86 ESBL SK1	CTX-M-15	B1	155	58		■											
	OW87 ESBL SK1	CTX-M-15	B1	155	58		■											
	OW3 ESBL SK1	SHV-12	B1		359	■												
	OW10 ESBL SK2	CTX-M-1	B1		5049	■												
	OW14 ESBL SK2	CTX-M-15	B1		295	■						■						
	OW18 ESBL SK1	CTX-M-15	B1		940													
	OW37 ESBL SK1	CTX-M-1	B1		1403													
	OW38 ESBL SK1	CTX-M-3	B1		1642													
	OW63 ESBL SK1	CTX-M-1	B1		3995	■												
	OW65 ESBL SK1	CTX-M-15	B1		517								■					

	OW67 ESBL SK1	CTX-M-15	B1		517
	OW4 ESBL SK2	CTX-M-27	B2		131
	OW14 ESBL SK1	CTX-M-14	B2		131
	OW15 ESBL SK1	CTX-M-27	B2		131
	OW15 ESBL SK2	CTX-M-15	B2		131
	OW18 ESBL SK2	CTX-M-15	B2		131
	OW77 ESBL SK1	CTX-M-15	B2		131
	OW86 ESBL SK2	CTX-M-27	B2		131
	OW89 ESBL SK1	CTX-M-15	B2		131
	OW91 ESBL SK1	CTX-M-14	B2		5055
	OW63 ESBL SK2	CTX-M-15	D	31	31
	OW64 ESBL SK2	CTX-M-15	D	38	38
	OW5 ESBL SK1	CTX-M-14	D	38	38
	OW2 ESBL SK1	CTX-M-15	D	69	69
	OW6 ESBL SK2	CTX-M-15	D	69	69
	OW50 E SK1	CTX-M-15	D	405	405
	OW1 CRE SK2	CTX-M-15	D	648	648
	OW15 CRE SK1	CTX-M-15	D	648	648
	OW18 CRE SK1	CTX-M-15	D	648	648
	OW8 ESBL SK3	CTX-M-27	D		1722
	OW54 ESBL SK1	CTX-M-55	D		5050
	OW66 ESBL SK2	CTX-M-15	D		5051
	OW77 ESBL SK2	CTX-M-15	D		5052
freshwater fish (n=33)/2013	F88v	CTX-M-1	A	10	10
	F105	CTX-M-15	A	10	10
	F140a	CTX-M-15	A	10	10
	F139	CTX-M-15	A	10	617
	F186	CTX-M-1	A	23	23
	F86b	CTX-M-15	A		1788
	F140b	CTX-M-15	A		3478
	F63	CTX-M-15	B1	155	155
	F91b	CTX-M-15	B1	155	155
	F109b	CTX-M-14	B1	155	155
	F109 violet same	CTX-M-14	B1	155	155
	F172a	CTX-M-15	B1	155	155
	F126	CTX-M-1	B1		1586
	F175b	CTX-M-15	B1		4695
	F88t	CTX-M-14	B2		131
	F95t	CTX-M-15	B2		131
	F110	CTX-M-27	B2		131
	F121	CTX-M-27	B2		131
	F136	CTX-M-15	B2		131
	F173	CTX-M-27	B2		131
	F175a	CTX-M-27	B2		131
	F172b	CTX-M-27	B2		2245

	F144b	CTX-M-27	B2		4226	
	F89	CTX-M-27	D	38	38	
	F132	CTX-M-14	D	38	38	
	F144a	CTX-M-24	D	38	38	
	F95b	CTX-M-14	D	69	69	
	F34 not in paper	CTX-M-1	D		394	
	F77	CTX-M-1	D		1462	
	F188	CTX-M-15	D		4692	
	F146	CTX-M-24	D		4693	
	F154	CTX-M-15	D		4694	
	F191a	SHV-12	D		4696	
vegetables (n=26)/2014	E26SK1	CTX-M-55	A	10	10	
	23SK2	CTX-M-55	A	10	48	
	ESBL DR28	CTX-M-65	A	10	167	
	ESBL H226 B	CTX-M-55	A	10	167	
	ESBL H238 V	CTX-M-15	A	23	410	
	49SK2	CTX-M-15	A	86	641	
	33SK1	CTX-M-55	A	226	226	
	E5	CTX-M-14	A		3696	
	23SK1	CTX-M-14	B1	155	58	
	40SK2	CTX-M-65	B1	155	58	
	E37SK2	CTX-M-1	B1	155	155	
	E49SK1b	CTX-M-15	B1	155	155	
	E49SK2b	CTX-M-15	B1	205	443	
	46SK2	CTX-M-15	B1		1081	
	ESBL DR47B	SHV-12	B1		1656	
	ESBL H239 V	CTX-M-14	B1		4679	
	ESBL H241 B	CTX-M-55	B1		4680	
	E37SK2.1	CTX-M-15	B1		4681	
	37SK1	CTX-M-15	B1		4682	
	2SK1	CTX-M-65	B1		4683	
	54SK2	CTX-M-15	B1		4684	
	ESBL DR06	CTX-M-15	B2		131	
	E3SK2	CTX-M-14	D	38	38	
	ESBL DR26	CTX-M-14	D	38	38	
	ESBL H226 L	CTX-M-55	D	31	393	
	ESBL DR45	CTX-M-15	D	405	405	
Livestock (n=28)/2009-2014	92-chicken	CTX-M-1	A	10	10	
	HV297.1	CTX-M-1	A	10	10	
	HV364.1	CTX-M-1	A	10	10	
	HV420.1	CTX-M-1	A	10	752	
	HV403.1	CTX-M-1	A		1112	
	72-pig	CTX-M-1	A		4447	
	128-calf	CTX-M-1	A		4701	
	59-chicken	CTX-M-1	B1	155	155	

	65-pig	CTX-M-1	B1	101	101	
	HV228	CTX-M-1	B1	101	101	
	HV295.1	CTX-M-1	B1		109	
	HV292.1	CTX-M-1	B1	446	602	
	49-chicken	CTX-M-1	B1	446	604	
	17-chicken	CTX-M-1	B1		683	
	HV84.1	CTX-M-1	B1		1056	
	HV114.1	CTX-M-1	B1		1056	
	HV183	CTX-M-1	B1		1056	
	HV337.1	CTX-M-1	B1		1056	
	HV369.1	CTX-M-1	B1		1056	
	HV226	CTX-M-1	B1		1146	
	23-chicken	TEM-52	B1		1389	
	60-chicken	CTX-M-1	B1		3174	
	HV359.1	CTX-M-1	B2		355	
	64-pig	CTX-M-1	D	350	57	
	HV290.1	CTX-M-1	D		117	
	2-lamb	CTX-M-1	D		295	
	HV300	CTX-M-1	D		1158	
	HV338.1	CTX-M-1	D		1629	
Chicken retail meat (n=13)/2012	PB11	CTX-M-1	A	10	93	
	PB22	CTX-M-1	A	23	88	
	PB34	CTX-M-1	A		4689	
	PB24	CTX-M-1	B1	155	58	
	PB10	CTX-M-1	B1	155	155	
	PB1	CTX-M-1	B1		1056	
	PB5	CTX-M-1	B1		1056	
	PB21	CTX-M-1	B2		1170	
	PB8	CTX-M-1	D	350	57	
	PB7	CTX-M-1	D		117	
	PB29	CTX-M-1	D		363	
	PB15	CTX-M-1	D		2309	
	PB31	CTX-M-1	D		4688	
Healthy humans (n=34)/2010	2018-human	CTX-M-1	A	10	10	
	1559-human	CTX-M-1	A	10	10	
	1519-human	CTX-M-1	A	10	10	
	1348-human	CTX-M-2	A	10	48	
	1582-human	CTX-M-1	A	10	93	
	2291-human	CTX-M-1	A	10	1638	
	1027-human	CTX-M-15	A		361	
	506-human	CTX-M-15	A		5044	
	2238-human	CTX-M-1	B1	86	453	
	1507-human	CTX-M-15	B1	205	443	
	2290-human	CTX-M-1	B1	446	602	
	150-human	CTX-M-15	B1	590	590	

	1038-human	CTX-M-1	B1		5056	
	2294-human	CTX-M-2	B1		129	
	2017-human	CTX-M-15	B1		940	
	1877-human	CTX-M-14	B2	95	95	
	1495-human	CTX-M-14	B2	131	131	
	490-human	SHV-12	B2		219	
	2200-human	CTX-M-15	B2		1193	
	1545-human	CTX-M-14	D	38	38	
	2241-human	CTX-M-14	D	69	69	
	2242-human	CTX-M-14	D	69	69	
	1887-human	CTX-M-15	D	69	69	
	2332-human	CTX-M-1	D	350	57	
	2333-human	CTX-M-1	D	350	57	
	503-human	CTX-M-15	D	405	405	
	171-human	CTX-M-15	D	405	405	
	2310-human	CTX-M-15	D	648	648	
	1866-human	CTX-M-15	D	648	648	
	1330-human	CTX-M-15	D	648	648	
	1024-human	CTX-M-15	D	648	648	
	1999-human	CTX-M-14	D		117	
	2225-human	CTX-M-15	D		501	
	1018-human	CTX-M-14	D		5054	
Primary care patients (n=13)/2012	HC192	CTX-M-1	A	10	10	
	HC267	CTX-M-14	A	10	10	
	HC121	CTX-M-15	A		4690	
	HC122	CTX-M-15	A		4690	
	HC270	CTX-M-1	B1		962	
	HC14B	CTX-M-14	B2		131	
	HC56	CTX-M-27	B2		131	
	HC111	CTX-M-27	B2		131	
	HC171	CTX-M-15	B2		131	
	HC247	CTX-M-14	D		362	
	HC200	CTX-M-15	D	405	405	
	HC264	CTX-M-27	D		2142	
	HC11	SHV-12	D		2142	

Figure S1: Sources, strain identities, extended-spectrum ß-lactamases (ESBL), phylogenetic groups, clonal complexes, sequence types, uropathogenic and enteric pathogenic virulence factors (VF) in *Escherichia coli* isolated from surface waters (Zurfluh et al., 2013), freshwater fish (Abgottspon et al., 2014a), fresh vegetables (Zurfluh et al., 2015), livestock (Geser et al, 2012; Zurfluh et al., 2014), retail chicken meat (Abgottspon et al., 2014b), healthy humans (Geser et al., 2012) and primary care patients (Nüesch-Inderbinen et al., 2013). Black squares indicate a feature present in that isolate. Grey squares denote features that are absent in the corresponding bacterial isolate. Abbreviations: MLST, multilocus sequence type; CC, clonal complex; UPEC, uropathogenic *E. coli*; EAEC, enteroaggregative *E. coli*; EPEC, enteropathogenic *E.coli*; ETEC, enterotoxigenic *E. coli*; STEC, shiga toxin-producing *E. coli*; EIEC, enteroinvasive *E. coli*.

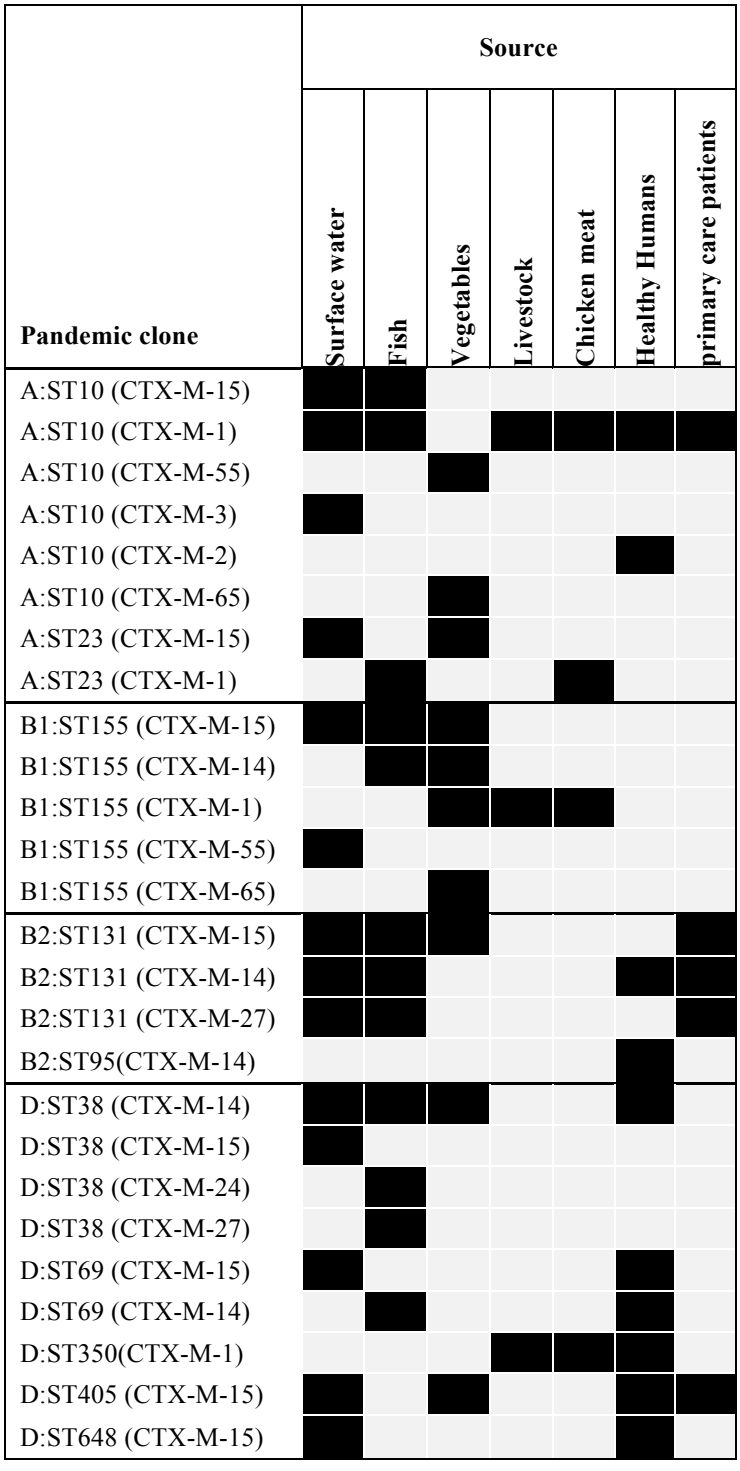


Figure 1: Occurrence and source overlaps of pandemic clones detected in surface waters, freshwater fish, fresh vegetables, livestock, retail chicken meat, healthy humans and primary care patients. Black squares indicate the presence of a clone in a source. Grey squares denote the absence of a clone in a source.

Table 1: Distribution of virulence factors (VF) among 207 ESBL-producing *E. coli* isolated from surface water, freshwater fish, fresh vegetables, livestock, retail chicken meat, healthy humans and primary care patients.

Description of gene or marker[a]	Gene or marker[b]	Prevalence by source[c]							
		Surface water (n=60)	Fish (n=33)	Vegetables (n=26)	Livestock (n=28)	Chicken meat (n=13)	Healthy Humans (n=34)	primary care patients (n=13)	Total (n=207)
UPEC-associated VFs									
Lipoprotein involved in serum resistance	*TraT*	35 (58.3%)	23 (69.7%)	15 (57.7%)	15 (53.6%)	10 (76.9%)	**8** (23.5%)	8 (61.5%)	114 (55%)
Ferric yersiniabactin uptake protein	*fyuA*	31 (51.6%)	21 (63.6%)	10 (38.5%)	**3** (10.7%)	5 (38.5%)	20 (58.8%)	9 (69.2%)	100 (48.3%)
Outer membrane hemin receptor	*chuA*	22 (10.6%)	19 (56.6%)	5 (19.2%)	**6** (21.4%)	6 (46.2%)	19 (55.9%)	8 (61.5%)	85 (41%)
Right-hand terminus of pathogenicity island from *E. coli* strain CFT073 (Kao et al., 1997)	PAI	18 (30%)	11(33.3%)	**1** (3.8 %)	10 (35.7%)	2 (15.4%)	**26** (76.5%)	4 (30.8%)	72 (34.7%)
Major subunit of a putative chaperone-usher fimbria	*yfcv*	14 (23.3%)	10 (30.3%)	1 (3.8%)	2 (7.1%)	2 (15.4%)	7 (20.6%)	4 (30.8%)	40 (19.3%)
IPEC-associated VFs									
Transcriptional activator of aggregative adherence fimbria I	*aggR* (EAEC)	**5** (8.3%)	1 (3%)	1 (3.8%)	—[d]	—	—	1 (7.7%)	8 (3.9%)
Intimin (attaching and effacing protein)	*eae* (EPEC)	2 (3.3%)	—	1 (3.8%)	1 (3.6%)	—	1 (2.9%)	—	5 (2.4%)
Human heat stable enterotoxin	STh (ETEC)	1 (1.6 %)	—	—	—	—	—	—	1 (0.5%)
Porcine heat stable enterotoxin	STp (ETEC)	—	**2** (6%)	—	—	—	—	—	2 (1%)
Heat-labile enterotoxin	LT (ETEC)	—	**2** (6%)	—	—	—	—	—	2 (1%)

[a] *vat*, *stx1*, *stx2*, and *ipaH* genes were not identified in any of the isolates.
[b] UPEC, uropathogenic *E. coli*; IPEC, intestinal pathogenic *E. coli*; EAEC, enteroaggregative *E. coli*; EPEC, enteropathogenic *E.coli*; ETEC, enterotoxigenic *E. coli*; STEC, shiga toxin-producing *E. coli*; EIEC, enteroinvasive *E. coli*.
[c] statistically significant values ($p < 0.005$) are indicated in underlined bold type.
[d] not detected.

Table 2: Distribution of VFs among the phylogenetic groups of 207 ESBL-producing *E. coli* isolated from surface water, freshwater fish, fresh vegetables, livestock, retail chicken meat, healthy humans and primary care patients.

Gene or marker[a,b]	**Prevalence by phylogenetic group**			
	A (n=62)	**B1 (n=60)**	**B2 (n=29)**	**D (n=56)**
UPEC-associated VFs				
TraT (UPEC)	25 (40.3%)	30 (50)	23 (79.3%)	36 (64.3%)
fyuA (UPEC)	21 (33.9%)	7 (11.1%)	28 (96.6%)	44 (78.6%)
chuA (UPEC)	—[c]	—	29 (100%)	56 (100%)
PAI (UPEC)	12 (19.4%)	10 (16.6%)	28 (96.6%)	22 (39.3%)
yfcv (UPEC)	1 (1.6%)	1 (1.6%)	27 (93.1%)	11 (19.6%)
IPEC-associated VFs				
aggR (EAEC)	3 (4.8%)	1 (1.6%)	—	4 (7.1%)
eae (EPEC)	1 (1.6%)	4 (6.6%)	—	—
STh (ETEC)	1 (1.6%)	—	—	—
STp (ETEC)	—	2 (3.3%)	—	—
LT (ETEC)	—	2 (3.3%)	—	—

[a] UPEC, uropathogenic *E. coli*; IPEC, intestinal pathogenic *E. coli*; EPEC, enteropathogenic *E.coli*; ETEC, enterotoxigenic *E. coli*; STEC, shiga toxin-producing *E. coli*; EIEC, enteroinvasive *E. coli*.
[b] *vat*, *stx1*, *stx2*, and *ipaH* genes were not identified in any of the isolates.
[c] not detected.

Table S1: Distribution and median aggregate VF scores of phylogenetic groups and of ESBL-producing pandemic clones among the sources

Phylogroup or clone	Aggregate VF score median (range)	Prevalence by source[a]							
		Surface water (n=60)	Fish (n=33)	Vegetables (n=26)	Livestock (n=28)	Chicken meat (n=13)	Healthy Humans (n=34)	Primary care patients (n=13)	Total (n=207)
Phylogroup A	1 (0-3)	25	7	8	7	3	8	4	62
clone									
A:ST10 (n=28)	1 (0-3)								28
A:ST10 (CTX-M-15)		**5**	3	0	0	0	0	0	8
A:ST10 (CTX-M-1)		1	1	0	4	1	**5**	1	13
A:ST10 (CTX-M-55)		0	0	**3**	0	0	0	0	3
A:ST10 (CTX-M-3)		2	0	0	0	0	0	0	2
A:ST10 (CTX-M-2)		0	0	0	0	0	1	0	1
A:ST10 (CTX-M-65)		0	0	1	0	0	0	0	1
A:ST23 (n=7)	1 (0-1)								
A:ST23 (CTX-M-15)		**4**	0	1	0	0	0	0	5
A:ST23 (CTX-M-1)		0	1	0	0	1	0	0	2
Phylogroup B1	1 (0-2)	13	7	13	**15**	4	7	1	60
clone									
B1:ST155 (n=15)	1 (0-2)								
B1:ST155 (CTX-M-15)		2	3	1	0	0	0	0	6
B1:ST155 (CTX-M-14)		0	2	1	0	0	0	0	3
B1:ST155 (CTX-M-1)		0	0	1	1	**2**	0	0	4
B1:ST155 (CTX-M-55)		1	0	0	0	0	0	0	1
B1:ST155 (CTX-M-65)		0	0	1	0	0	0	0	1
Phylogroup B2	5 (2-5)	9	9	1	1	1	4	4	29
clone									
B2:ST131 (n=21)	5 (3-5)								
B2:ST131 (CTX-M-15)		4	2	1	0	0	0	1	8
B2:ST131 (CTX-M-14)		1	1	0	0	0	1	1	4
B2:ST131 (CTX-M-27)		3	**4**	0	0	0	0	2	9
B2:ST95 (n=1)	5 (5)								1
B2:ST95(CTX-M-14)		0	0	0	0	0	1	0	
Phylogroup D	3 (1-5)	13	10	4	5	5	15	4	56
clone									
D: ST38 (n=8)	3 (2-3)								
D:ST38 (CTX-M-14)		1	1	2	0	0	1	0	5
D:ST38 (CTX-M-15)		1	0	0	0	0	0	0	1
D:ST38 (CTX-M-24)		0	1	0	0	0	0	0	1
D:ST38 (CTX-M-27)		0	1	0	0	0	0	0	1
D:ST69 (n=6)	3 (2-3)								
D:ST69 (CTX-M-15)		2	0	0	0	0	1	0	3
D:ST69 (CTX-M-14)		0	1	0	0	0	2	0	3
D:ST350 (n=4)									
D:ST350(CTX-M-1)		0	0	0	1	1	2	0	4
D:ST405 (n=5)	3 (3-4)								
D:ST405 (CTX-M-15)		1	0	1	0	0	2	1	5
D:ST648 (n=7)	5 (4-5)								
D:ST648 (CTX-M-15)		3	0	0	0	0	**4**	0	7

[a] statistically significant values ($p < 0.005$) are indicated in underlined bold type

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